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Redescription of *Monticellia magna* (Rego, dos Santos & Silva, 1974) (Eucestoda: Monticelliidae) parasite of *Pimelodus* spp. (Pisces: Siluriformes) from Argentina, and morphological study of microtriches

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Redescription of *Monticellia magna* (Rego, dos Santos & Silva, 1974) (Eucestoda: Monticelliidae) parasite of *Pimelodus* spp. (Pisces: Siluriformes) from Argentina, and morphological study of microtriches. - *Monticellia magna* (Rego, dos Santos & Silva, 1974) is redescribed and the microtriches and their distribution are studied for the first time. This species is characterised by the following combination of characters: (1) vagina anterior to cirrus pouch; (2) muscular asymmetrical sphincter present; (3) testes in one layer and in two fields connected anteriorly and posteriorly; (4) vitelline follicles distributed cortical, paramuscular and a few follicles medullary; (5) internal longitudinal musculature strongly developed; and (6) scolex with filiform microtriches in apical region, filiform and spiniform microtriches in central cavity, marginal ring and nonadherent surface of suckers, and spiniform microtriches in neck and immature proglottides. The species parasitised fishes of *Pimelodus* spp. Strict specificity of South American proteocephalids for their hosts is placed into consideration since recently new host records have been reported, especially for hosts commercially exploited.

Key-words: microtriches - Proteocephalidea - *Monticellia magna* - Pimelodidae - host specificity - Argentina.

INTRODUCTION

Monticellia magna (Rego, dos Santos & Silva, 1974) was originally described from *Pimelodus clarias* (Bloch, 1782) (junior synonym) (= *P. blochii* Valenciennes, 1840, original combination) as *Nomimoscolex magna*. However, de Chambrier & Vaucher (1997) studied the type material and found it composed by specimens that belong to two different genera, *Proteocephalus* and *Monticellia*; and transferred *N. magna* to *Monticellia*.

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Latter, Rego & Pavanelli (1992) described *Monticellia loyolai* from *Pimelodus maculatus* Lacépède, 1803. De Chambrier & Vaucher (1999) synonymized this species with *M. magna* based on the study of the type material and new material collected in Paraguay. Eventhough, these authors exposed remarkable and distinctive characters for *M. magna*, and did not present drawings of this species.

During a survey of proteocephalidean cestodes from freshwater teleost fishes in Argentina, specimens of *M. magna* were collected from *Pimelodus albicans* (Valenciennes, 1840), *P. argenteus* Perugia, 1891, and *P. maculatus*. *M. magna* is described in detail for the first time in this paper, based on type and the new material. The surface of the tegument of the scolex and portions of the strobila were studied using scanning electron microscopy (SEM).

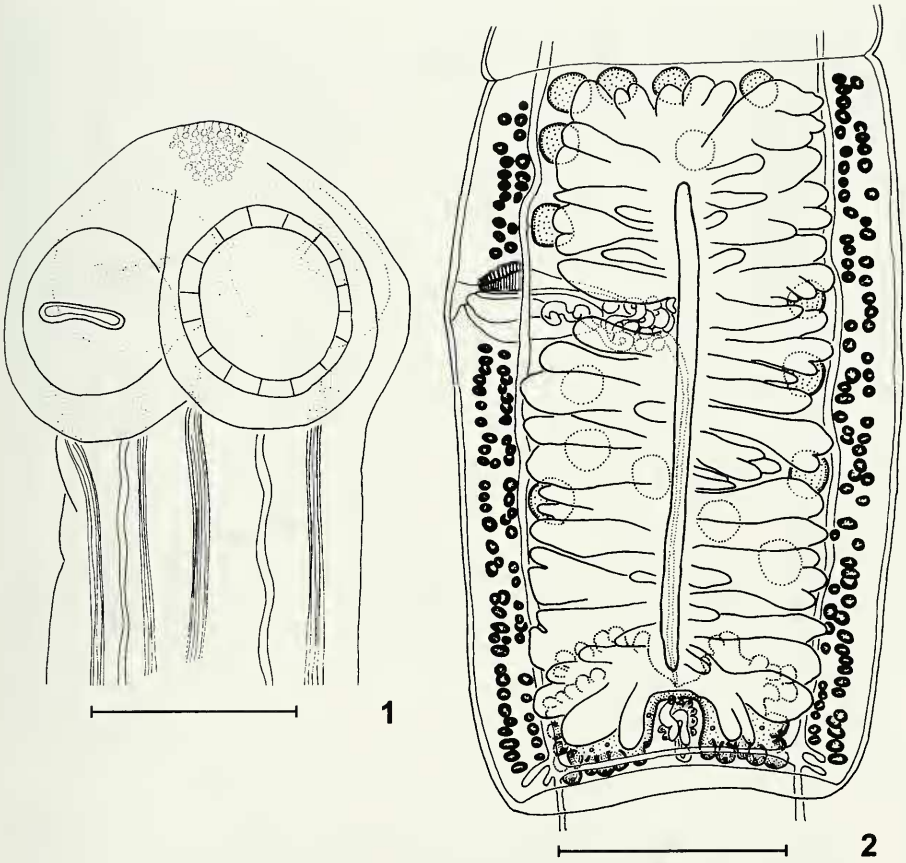
MATERIAL AND METHODS

Eighty specimens of *P. albicans*, 6 of *P. argenteus* and 212 of *P. maculatus* from Colastiné, La Plata, and Paraná rivers were examined for helminths. Worms found in the intestine were isolated and fixed in hot 4% v/v formaldehyde solution and stored in 75% v/v ethanol. Entire tapeworms were stained with Langeron´s alcoholic chlorhydric carmine (Langeron, 1949), differentiated in acid ethanol, dehydrated through a gradual ethanol series, cleared in beechwood creosote and mounted in Canada balsam. Thick transverse hand-cutting serial sections of proglottides were stained following the same procedure. Eggs were mounted in distilled water, after fixation for drawing. Three specimens were prepared for SEM as follows: post-fixed in 1% osmium tetroxide, dried with tetrametylsilane (Analyticals®, Carlo Erba), mounted on stubs with adhesive tape, sputter coated with gold in a Thermo VG Scientific Polaron SC 7630 and examined with a Philips XL 30 scanning electron microscope. Microthrix density values (D) were obtained by counting microtriches from randomly selected areas of 1 μm^2. Voucher specimens of *M. magna* from Argentina were deposited at Colección Parasitológica del Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires, Argentina (MACN-Pa), and at the Natural History Museum, Geneva, Switzerland (MHNG). Syntypes of *M. magna* (Rego, dos Santos & Silva, 1974) and *M. loyolai* (Pavanelli & Machado dos Santos, 1992) from Helminthological Collection of the Instituto Oswaldo Cruz (CHIOC) were also studied. The information on taxonomic classification of fishes was obtained from FishBase Online (www.fishbase.org). All measurements are given in micrometers, unless otherwise stated, with the range followed by the mean, the standard deviation and the number of measurements (n) in parentheses. Measurements of microtriches were determined from photomicrographs. Illustrations were made with the aid of a camera lucida using Nomarski interference contrast in a Zeiss Axioscope microscope.

RESULTS

Monticellia magna (Rego, dos Santos & Silva, 1974) Figs 1-14
Nomimoscolex magna Rego, dos Santos & Silva, 1974
Monticellia loyolai Pavanelli & Machado dos Santos, 1992

Type host: *Pimelodus clarias* (Bloch, 1782) (junior synonym) (= *P. blochii* Valenciennes, 1840, original combination).



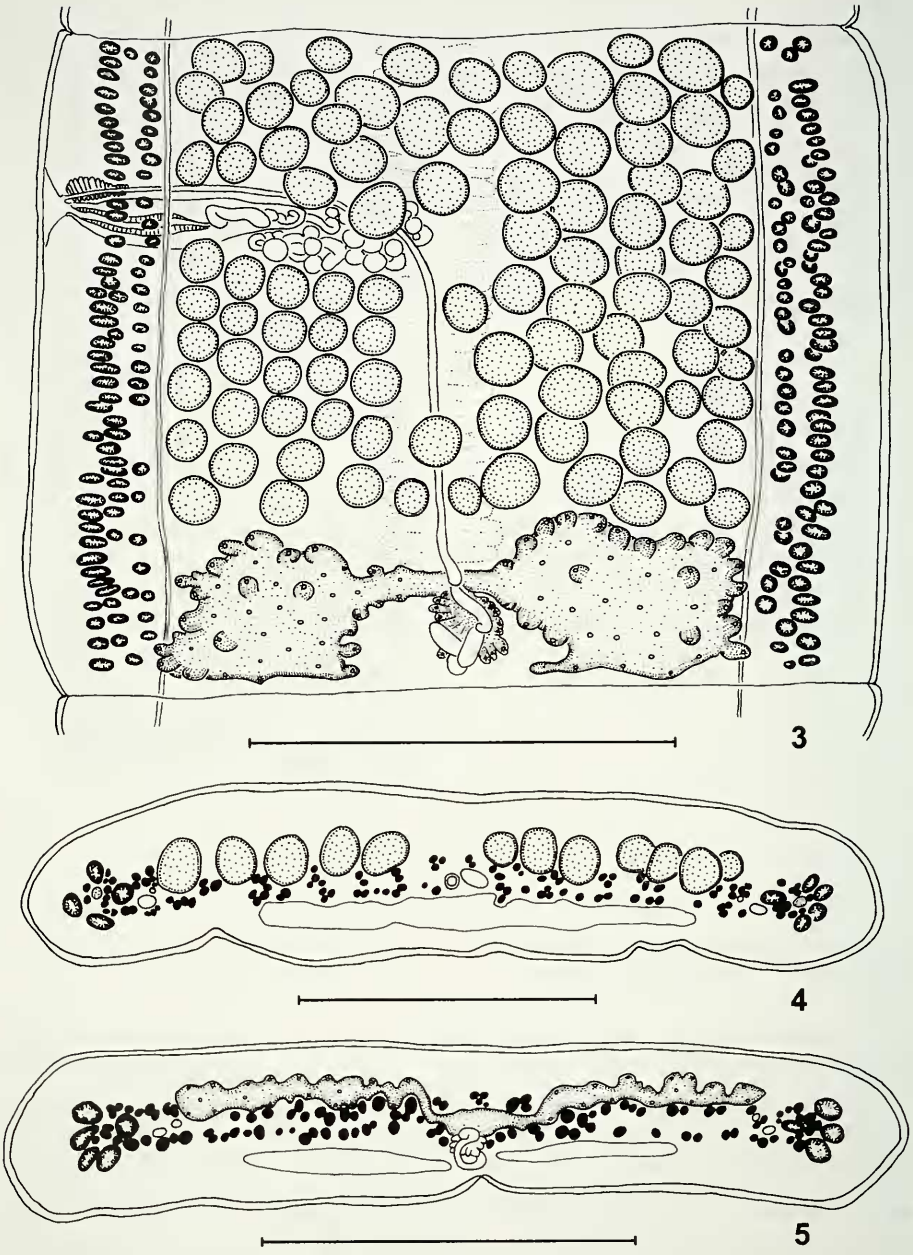
FIGS 1-2

Monticellia magna (Rego, dos Santos & Silva, 1974). 1. Scolex, apical region showing unicellular gland cells. 2. Gravid proglottis, ventral view. Scale-bars: 1 = 250 µm; 2 = 500 µm.

Additional hosts: *Pimelodus albicans* (Valenciennes, 1840), vernacular name: moncholo, bagre blanco; *Pimelodus argenteus* Perugia, 1891, vernacular name: bagre blanco; *Pimelodus maculatus* Lacépède, 1803, vernacular name: bagre amarillo (Siluriformes: Pimelodidae).

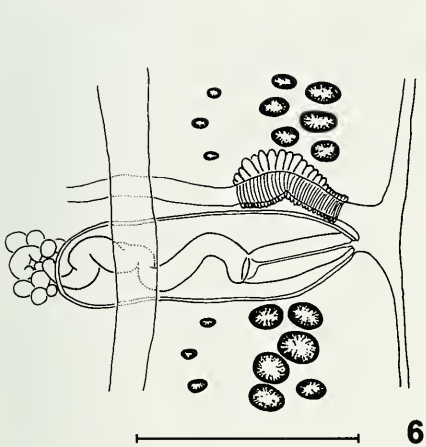
Material studied: **Argentina**: 1) Buenos Aires Province, Buenos Aires Port, La Plata river (34°37'S, 58°22'W), MACN–Pa 419/1-3, collected from *P. albicans* on 23/10/1989, and MACN-Pa 423/1-2 collected from *P. maculatus* on 10/09/1994. 2) Santa Fé Province, Santo Tomé City, Colastiné river (tributary of Paraná river) (31°40'S, 60°46'W), MACN–Pa 423/3-4 and MNHG 34660 INVE, collected from *P. maculatus* on 15/02/2002 and 31/07/2001 respectively, and MACN-Pa 424/1-2 and MNHG 34661 INVE collected from *P. argenteus* on 13/12/2002. Type specimens from **Brazil**: 1) Mato Grosso State, Esperanza Port, syntypes *M. magna* (Rego, dos Santos & Silva, 1974) CHIOC 31049 a-c, 2 contracted specimens; CHIOC 33137 (= 4476), fragment of strobila; CHIOC 33139 (= 4480), fragment of strobila. 2) Paraná State, Paraná river, *M. loyolai* (Pavanelli & Machado dos Santos, 1992), CHIOC 32715 (holotype); CHIOC 32716 c-d and 32717 a-b transverse sections of proglottides (paratypes).

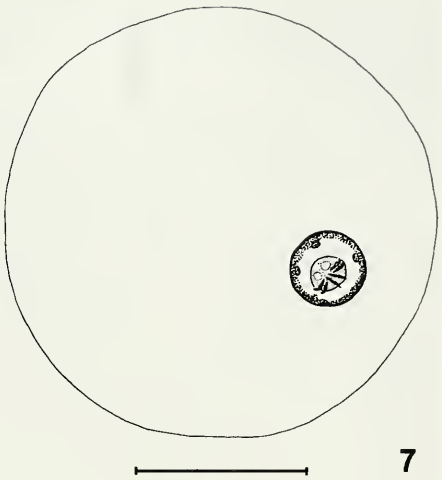
Prevalence: 40% (80 *P. albicans* examined), 33% (6 *P. argenteus* examined), 70% (212 *P. maculatus* examined).



FIGS 3-5

Monticellia magna (Rego, dos Santos & Silva, 1974). 3. Mature proglottis, dorsal view. 4-5. Transverse sections of proglottides showing internal longitudinal musculature, and topography of the genitalia. 4. Transverse section anterior to ovary. 5. Transverse section at level of ovary. Scale-bars: 3-5 = 500 µm.





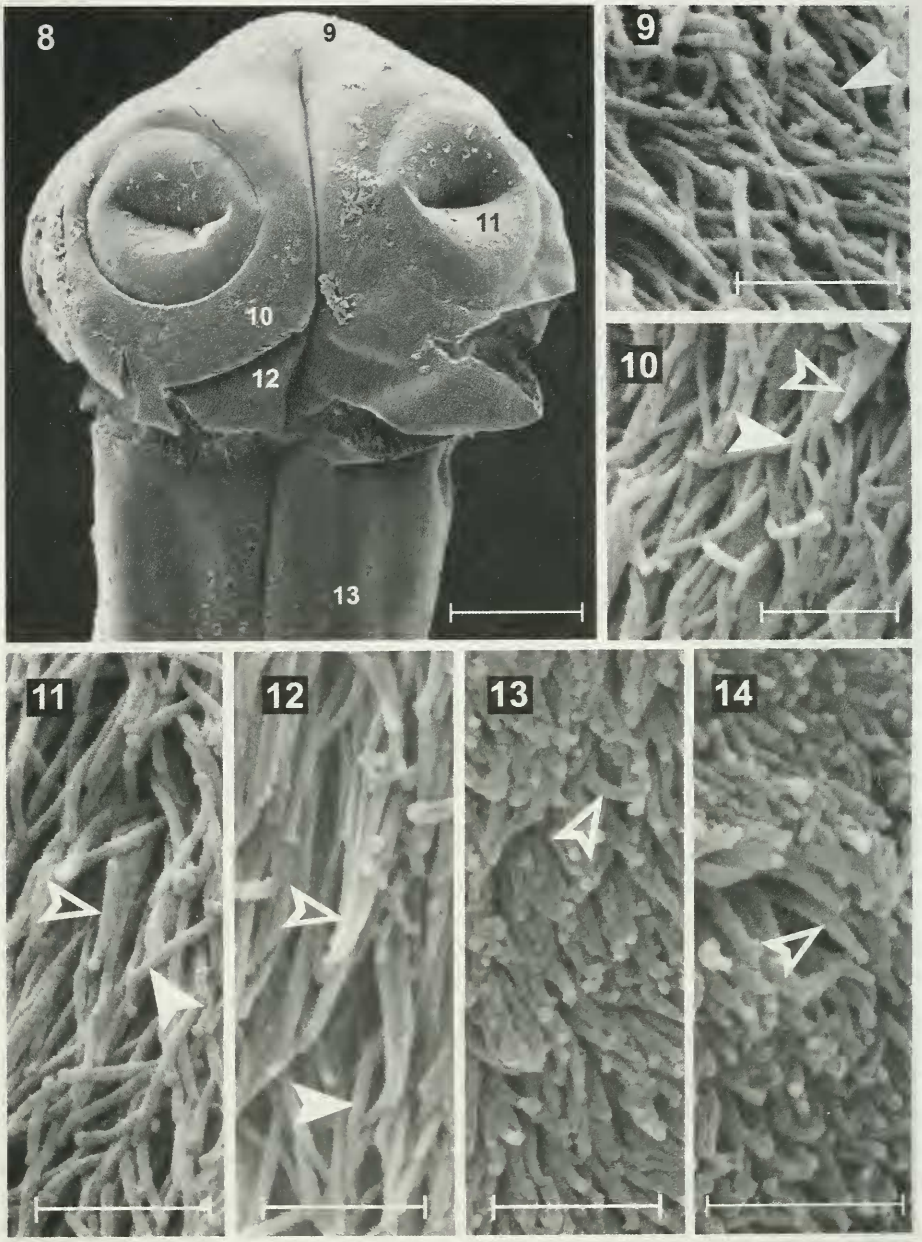
FIGS 6-7

Monticellia magna (Rego, dos Santos & Silva, 1974). 6. Detail of cirrus pouch and vagina showing the asymmetrical sphincter. 7. Eggs drawn in distilled water, after fixation. Scale-bars: 6 = 200 µm; 7 = 100 µm.

Intensity of infection: 2-20 worms per fish.
Site of infection: anterior and middle part of intestine.

Description (based on 15 specimens and measurements on 9 specimens from Argentina): Proteocephalidea, Monticelliidae, Monticelliinae. Testes, ovary and uterus cortical. Vitelline follicles partly in the cortex and partly in the medulla. Medium size worms, 23-125 mm, flattened dorsoventrally. Strobila acraspedote, anapolytic, consisting of 53-125 (n = 9) proglottides: 20-60 immature, 8-22 mature, and 12-60 gravid.

Scolex wider than proliferation zone (Figs 1, 8), 480-830 (582 ± 58, n = 9) wide. Apical organ absent, numerous spherical-shaped glandular cells with granular inclusions, distributed in apical region. Apical region of scolex proper covered with densely packed filiform microtriches 1.1-1.3 (n = 4) long, 0.1 wide, D = 21-25 (n = 4) (Fig. 9). Suckers spherical to oval, unilobate, uniloculate, strongly muscular, 220-300 (246, n = 18) long, 160-300 (217) wide. Central cavity surface of suckers covered with filiform microtriches 0.8-1.2 (n = 5) long, 0.1 wide, interspersed with spiniform microtriches 0.9-1.1 (n = 6) long, 0.2-0.3 wide, D = 23 filiform : 1-2 spiniform (n = 4) (Fig. 11). Marginal ring surface of suckers covered with filiform microtriches 0.9-1.2 (n = 5) long, 0.1 wide, interspersed with spiniform microtriches 1.0-1.3 (n = 6) long, 0.2-0.3 wide, D = 14-20 filiform : 1-2 spiniform (n = 4) (Fig. 10). Nonadherent surface of suckers covered with filiform 1.0-1.2 (n = 5) long, 0.1 wide, interspersed with spiniform microtriches, 1.1-1.3 (n = 4) long, 0.2 wide, D = 8-14 filiform : 2-3 spiniform (n = 4) (Fig. 12). Proliferation zone (neck), 800-3000 (1422, n = 9) long, surface covered with spiniform microtriches, 0.6-0.8 (n = 4) long, 0.2 wide, D = 31-35 (n = 4) (Fig. 13).



FIGS 8-14. *Monticellia magna* (Rego, dos Santos & Silva, 1974) SEM micrographs. 8. Scolex in dorsoventral view, and positions of high magnification views for Figs 9-13. 9. Apical region surface, filiform microtriches. 10-12. Suckers: marginal ring, central cavity, and nonadherent surface, respectively, filiform microtriches interspersed with spiniform microtriches. 13. Proliferation zone surface, spiniform microtriches. 14. Immature proglottis surface, spiniform microtriches. Scale-bars: 8 = 100 µm; 9-12, 14 = 1 µm; 13 = 2 µm. Full arrows show filiform microtriches, empty arrows show spiniform microtriches.

Immature proglottides wider than long, 120-590 (338, n = 12) long, 430-800 (598) wide. Immature proglottides surface covered with spiniform microtriches 0.6-0.7 (n = 5) long, 0.2 wide, D = 38-43 (n = 4) (Fig. 14). The surface of proliferation zone and the immature proglottis is covered with spiniform microtriches, and they are the regions with higher densities (D = 31-35 and 38-43 respectively). Mature proglottides wider than long or longer than wide, 400-1010 (674 ± 154, n = 37) long, 880-2620 (1340 ± 547) wide (Fig. 3). Gravid proglottides wider than long or longer than wide, 500-2200 (1150 ± 373, n = 34) long, 870-2740 (1602 ± 621) wide (Fig. 2).

Internal longitudinal musculature strongly developed, forming thick fibre bundles, delimiting a reduced medulla. Osmoregulatory canals situated between testes and vittelline follicles. Ventral canal, 12-20 in diameter, with secondary osmoregulatory canals ending on ventral surface lateral to ovarian lobes. Dorsal canal, 5-10 in diameter (Figs 4, 5).

Testes cortical, total number 85-146 (106, n = 22) in mature proglottides, 40-100 (70, n = 18) in diameter; in one layer, in two fields connected anteriorly and posteriorly (Fig. 3). Occasionally, 1-3 testes overlapping vas deferens and distal part of cirrus pouch. Cirrus pouch pyriform with thin muscular wall, 210-320 (260 ± 36, n = 31) long, 40-85 (66 ± 13) wide; occupying 21-29% (24% ± 2, n = 31) of proglottis width in mature proglottides. Cirrus occupying about 30-62% of cirrus pouch length. Vas deferens coiled, 20-25 in diameter, usually not surpassing mid-line of body in mature proglottides. Genital pores irregularly alternating, situated anteriorly at 20-42% (28% ± 4, n = 31) of proglottis width.

Vagina anterior (99%) to cirrus pouch, 13-20 in diameter, strongly asymmetrical and muscular sphincter present (Fig. 6). Ovary cortical, with 2 lobulate lobes; occupying 56-74% (67% ± 4, n = 31) of proglottis width in mature proglottides.

Vitelline follicles cortical and paramuscular, with 1-2 follicles lying in medulla. Forming 2 lateral bands concentrated in mid-lateral region of proglottis, interrupted at cirrus pouch and vagina level on ventral side, reaching 98-100% of total proglottis length (Figs 3, 4, 5).

Uterine primordium stem and uterine branches cortical. Uterine branches occupying up to 70% of gravid proglottis width; 19-29 (22, n = 14) lateral branches opposite to cirrus pouch side, and 18-28 (20) on cirrus pouch side. Cortical uterine diverticula nearly completely overlap the ovary. Mature eggs released by a ventral longitudinal slit (Fig. 2). Eggs with thick hyaline outer envelope, 160-275 (228, n = 7) in diameter; embryophore , 35-48 (41, n = 7) in diameter; oncosphere 18-20 (n = 7) in diameter; hooks 10-12 long (Fig. 7).

DISCUSSION

The genus *Monticellia* La Rue, 1911 includes 11 species, all distributed in the Neotropical region: *M. amazonica* de Chambrier & Vaucher, 1997, *M. belavistensis* Pavanelli, Machado, Takemoto & dos Santos, 1994, *M. coryphicephala* (Monticelli, 1891), *M. dlouhyi* de Chambrier & Vaucher, 1999, *M. lenha* Woodland, 1933, *M. magna*, *M. mandi* (Pavanelli & Takemoto, 1996), *M. megacephala* Woodland, 1934, *M. ophisterni* Scholz, de Chambrier & Salgado-Maldonado, 2001, *M. spinulifera*, Woodland, 1935, and *M. ventrei* de Chambrier & Vaucher, 1999. *M. diesingii*

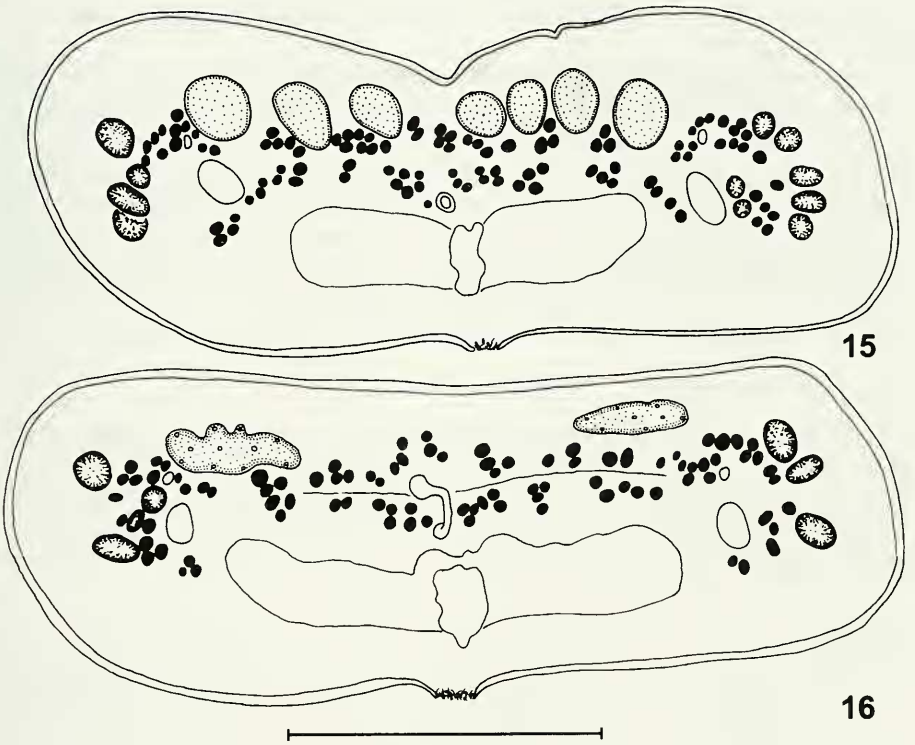
(Monticelli, 1891) and *M. macrocotylea* (Monticelli, 1892) are considered species inquirendae (Rego *et al.*, 1999).

Among the morpho-anatomical features studied in this redescription the following combination of characters are important to characterise *M. magna*: (1) vagina anterior to cirrus pouch; (2) muscular asymmetrical sphincter present; (3) testes in one layer and in two fields connected anteriorly and posteriorly; (4) vitelline follicles distributed cortical, paramuscular and a few follicles medullary; (5) internal longitudinal musculature strongly developed; and (6) scolex with filiform microtriches in apical region, filiform and spiniform microtriches in central cavity, marginal ring and nonadherent surface of suckers, and spiniform microtriches in proliferation zone and immature proglottides.

The syntypes of *M. magna* were studied and the conspecificity with the specimens from Argentina was confirmed. Transverse sections were not available from the material examined. The holotype and the paratypes of *Monticellia loyolai* (= *M. magna*) were also studied. The asymmetrical vaginal sphincter and uterine branches nearly completely overlapping the ovary were clearly observed in type specimens. The vitelline follicles are situated cortical, paramuscular, and medullary (Figs 15, 16). The same topography was observed in the specimens collected from *P. albicans*, *P. argenteus* and *P. maculatus* from Argentina, thus the conspecificity with *M. magna* was also confirmed.

In the genus *Monticellia*, only *M. spinulifera* has been partially examined with SEM (Rego, 1999). Even when this author studied the sucker at very low magnification, and the giant spiniform microtriches on the marginal ring of the sucker could be easily observed. However, from the photomicrograph it is not known if the spiniform microtriches are the only kind of microtriches on the marginal ring surface or if they are interspersed with other types of microtriches. Therefore, it is necessary to study in detail all the regions of the scolex proper to confirm the microthrix distribution of *M. spinulifera*. To date, in the Neotropical proteocephalids only *Nomimoscolex semenasae* Gil de Pertierra, 2002 (Monticelliidae, Zygobothriinae), and *M. magna* in this paper were completely analysed for the microthrix distribution with SEM.

Host-specificity varies widely among different taxa of fish helminths. Highly host-specific parasites are restricted to one host species and specificity declines as the number of suitable host species increases (Poulin, 1998). The South American proteocephalideans have been considered to be specific to one fish host species. In fact, a few examples of species having more than one final host were registered: (1) *Amazotaenia yvettae* de Chambrier, 2001 from *Brachyplatystoma filamentosum* Lichtenstein, 1819, and *B. vaillantii* Valenciennes, 1840; (2) *Choanoscolex abscissus* (Riggenbach, 1896) from *Zungaro zungaro* (Humboldt, 1821) [(= *Paulicea luetkeni* (Steindachner, 1876)], *Pseudoplatystoma corruscans* (Agassiz, 1829), *P. fasciatum* Linnaeus, 1766, and *Raphiodon vulpinus* Spix & Agassiz, 1829 (see Rego *et al.*, 1999); (3) *Harriscolex kaparari* (Woodland, 1934) from *P. corruscans*, and *P. tigrinum* (Valenciennes, 1840); (4) *Monticellia magna* from *Pimelodus albicans* (new record), *P. argenteus* (new record), *P. clarias*, and *P. maculatus*; (5) *M. ventrei* de Chambrier & Vaucher, 1999 from *Luciopimelodus pati* (Valenciennes, 1836) (new record), and *Pinirampus pirinampu* (Spix & Agassiz, 1829); (6) *Nomimoscolex microacetabula* Gil de Pertierra, 1995 from



FIGS 15-16

Monticellia magna (Rego, dos Santos & Silva, 1974), transverse sections of proglottides drawn from holotype CHIOC 32715a, showing internal longitudinal musculature, and topography of the genitalia. 15. Transverse section anterior to ovary. 16. Transverse section at level of ovary. Scale-bar: 15, 16 = 500 μm.

P. albicans, and *P. maculatus*; (7) *N. suspectus* Zehnder, de Chambrier, Vaucher & Mariaux, 2000 from *B. filamentosum*, *B. vaillantii*, and *Z. zungaro* [(= *B. flavicans* (Castelnau, 1885)]; (8) *Nupelia tomasi* de Chambrier & Vaucher, 1999 from *Trachelyopterus galeatus* (=*Parauchenipterus galeatus*) (Linnaeus, 1766), and *T. cf. striatulus* (= *P. striatulus*) (Steindachner, 1877); (9) *Peltidocotyle rugosa* Diesing, 1850 (see Zehnder & de Chambrier, 2000) from *P. corruscans*, *P. fasciatum*, and *P. tigrinum*; (10) *Peltidocotyle lenha* (Woodland, 1933) (see Zehnder & de Chambrier, 2000) from *Z. Zungaro*, and *Sorubimichthys planiceps* (Spix & Agassiz, 1829); and (11) *Proteocephalus microscopicus* Woodland, 1935 from *Cichla monoculus* Spix & Agassiz, 1831, and *Cichla ocellaris* Bloch & Schneider, 1801.

Poulin (1992, 1997) stated that high host-specificity can be an artefact of inadequate sampling, and among species of parasites of freshwater fishes sampling effort explains much of the variability in host-specificity. The number of South American proteocephalid species known parasitising more than one fish host might be correlated with a larger sampling effort. Among the fishes mentioned previously 81% are commercially exploited (*Cichla*, 2 species; *Brachyplatystoma*, 2 species; *Luciopi-*

melodus pati; *Pimelodus*, 3 species; *Pinirampus pirinampu*; *Pseudoplatystoma*, 3 species; *Z. zungaro*)

M. magna is widespread within *Pimelodus* spp., these host species are very frequent, and is common food among people living nearby the Argentinian rivers.

In this study accurate drawings of the scolex, mature and gravid proglottides, transverse sections of the proglottides at different levels, detail of the vaginal sphincter, eggs are revisited for the first, and the types of microtriches and their distribution are presented for the first time, the ranges of measurements and the mean are in agreement with the values given by de Chambrier & Vaucher (1999), differences were registered only for the number of uterine branches (18-36 vs 37-57 in this paper), and cirrus length/cirrus pouch length (30% vs 30-62% in this paper).

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